Breast cancer - new aspects of tumor biology: are calcitriol and cyclooxygenase-2 possible targets for breast cancer?

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Summary

Up until now there have been many advances in treatment options for breast cancers such as targeted therapies like monoclonal antibodies, tyrosine kinase inhibitors, mTOR antagonists, and vaccines. Despite these advances, there are still many more that warrant further exploration. Two of these targets might be the cyclooxygenase-2 (COX-2), the key enzyme required to convert arachidonic acid to prostaglandins, and calcitriol [$1,25(OH)_2D_3$] which is the biologically active form of vitamin D. Both calcitriol and the inhibition of COX-2 have shown antiproliferative and prodifferentiation, as well as pro-apoptotic effects in different malignancies in vitro and in vivo, and the key prostaglandin catabolic enzyme 15-hydroxyprostaglandin dehydrogenase (15-PGDH) is known to have tumor suppressor activity. Furthermore, the combination of calcitriol and nonsteroidal anti-inflammatory drugs (NSAIDs), such as non-selective and selective COX-2 inhibitors, acting synergistically to achieve significant cell growth inhibition in prostate cancer. Some epidemiological studies suggest that vitamin D confers a moderate benefit against breast cancer while most epidemiological studies presume that NSAIDs confer the same. Nevertheless there is growing body of evidence that COX-2 expression is a fundamental step in breast cancer carcinogenesis. To date, clinical trials have been conducted in patients with different malignancies using treatment strategies including COX-2 inhibitors and calcitriol and are showing partially encouraging results. The goal of this review is to shed light on the association between the prostaglandin as well as vitamin D metabolism relating to the incidence and therapy of breast cancers. Moreover, this review will also highlight potential treatment options, as well as extract any existing interactions between the two metabolisms.

Key words: Prostaglandin; Vitamin D; Calcitriol; Cyclooxygenase-2 (COX-2); Breast cancer.

Introduction

Currently, breast cancer is the most common malignancy in women. In the U.S. in 2005, approximately 211,240 patients were newly diagnosed with primary breast cancer and 58,490 women were diagnosed with ductal carcinoma in situ (DCIS). Of these, 58,490 deaths are estimated. Therefore breast cancer takes second place following only behind lung cancer [1-3]. Because of this, it is necessary to develop new strategies and treatment options that may improve the prognosis.

Besides the classic histo-pathological parameters used to estimate the prognosis of malignant diseases, the identification of additional molecular prognostic parameters would be very helpful in planning treatment by evaluating protein or messenger ribonucleic acid (mRNA) expression in tumor tissue. One of these potential molecular prognostic parameters might be the cyclooxygenase-2 (COX-2) [4, 5]. New treatment strategies using compounds that attack well defined proteins in the tumor require verification of the expression of these target proteins. Many similarities exist between tumor tissue and inflammatory modified tissue and normally, inflammatory reaction is self-limiting, however, in tumor tissue the inflammatory reaction is persistent. An increased angiogenesis and an elevated production of cytokines, chemokines, and proteases lead to good conditions for cell proliferation and invasion in the tumor tissue [6].

Targeted strategies might eliminate this inflammatory reaction that promotes tumor growth and tumorigenesis and there is already promising data regarding the use of COX-2-inhibitors. The antiproliferative effects of vitamin D may be another starting point; however the data on vitamin D intake or on the exertion of vitamin D analogs is occasionally inconsistent.

The important role that vitamin D and calcium adopt in the human metabolism was recognized as early as the 1920s as it was used to prevent bone disease and rickets which was widespread in children at that time [7]. In the last 20 years non-classical effects of vitamin D and its influence on physiology followed because it is potentially anticarcinogen impacts made it more and more interesting. Besides stable calcium-homeostasis by the renal expressed 1-a-hydroxylase functionality, extra-renal expressed 1-a-hydroxylase also is also known to have antiproliferative and immune-modulating features [8-10]. This fact has led to the development of new treatment strategies in the clinical use of $1,25(OH)_2D_3$ (calcitriol). The goal was to affect and treat cancer, psoriasis, autoimmune diseases, and host-graft-rejection [11-14]. Implementation of these

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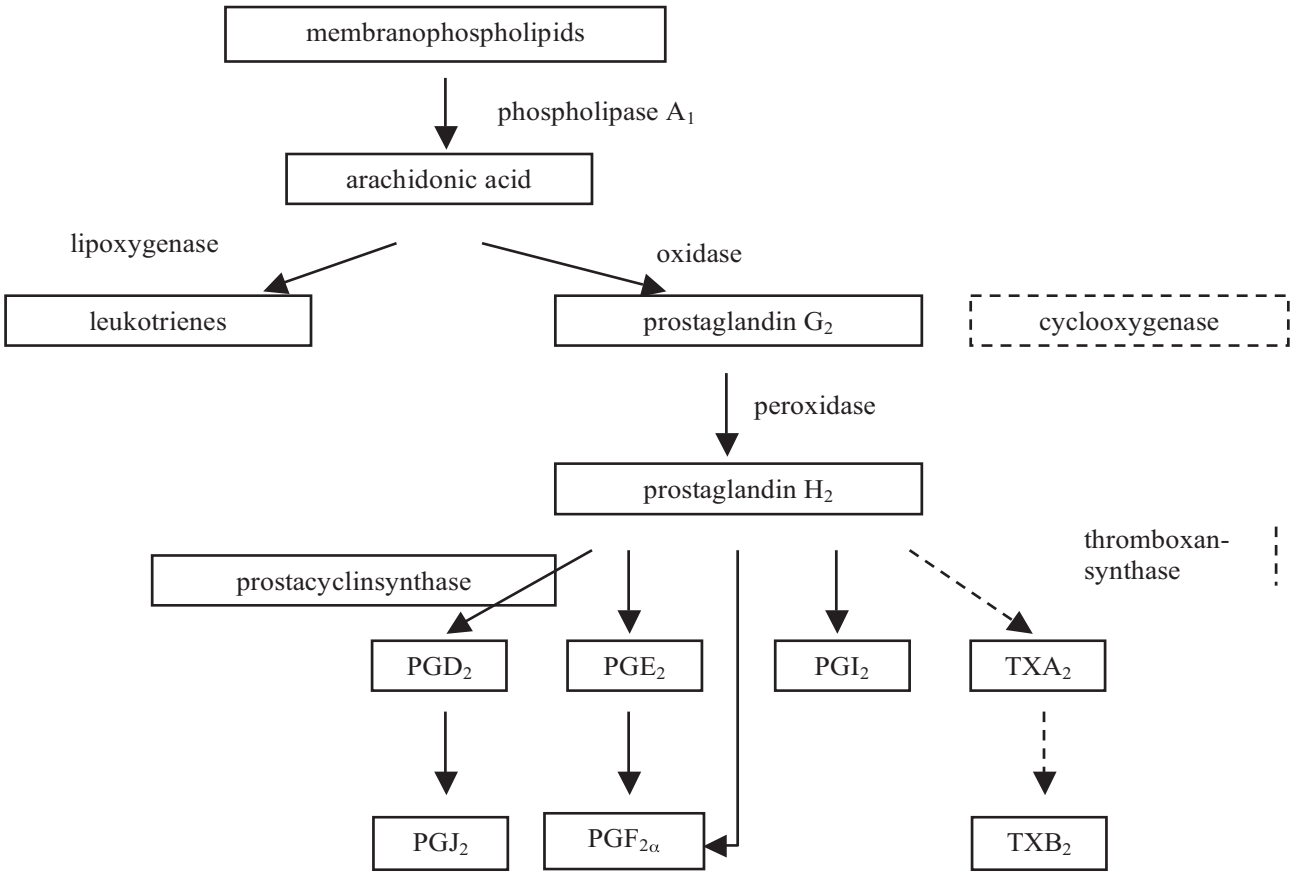

Figure 1. — Prostaglandin metabolism.

new treatment options in vivo was conspicuously hindered as $1,25(OH)_2D_3$ has a potentially hypercalcaemic side effect. Finally the application of synthetic $1,25(OH)_2D_3$ analogs led to several successful results due to its less calciotropic effects [15, 16]. The implementation of vitamin D, primarily in cancer and autoimmune diseases, appears to play a more preventative role as opposed to therapeutic [17, 18].

Observational studies showed an association between vitamin D intake and $25(OH)_2D_3$ plasma levels, as well as a reduced risk of breast cancer [19, 20]. Studies that tried to elucidate the correlation between sunlight and cancer prevention demonstrated that long sunlight exposure was associated with a low rate of primary breast cancer and consecutively a low mortality rate [21-24]. $1,25(OH)_2D_3$ is the biologically active form of vitamin D that binds as a ligand to the nuclear vitamin D receptor (VDR) of the genes that are important for vitamin D metabolism (1-a-hydroxylase, 24-hydroxylase) [25]. $1,25(OH)_2D_3$ and its analogs are able to inhibit the proliferation of breast cancer cells in vitro and in vivo [26-29].

Prostaglandin metabolism

The COX system consists of two different isoenzymes, COX-1 and COX-2. This system is an integral part of the prostaglandin syntethase complex and is involved controlling inflammatory processes (Figure 1). After transformation of arachidonic acid to prostaglandin G_2 (PGG_2), a glutathione dependent peroxidase converts PGG_2 to PGH_2 by an oxi- and peroxidation. PGH_2 acts as basic substrate for the synthesis of different prostaglandins by the microsomal and cytosolic prostaglandin synthase, which are tissue- and cell-specific. Based on the cellular enzyme setting, different prostaglandins are synthesized in different tissues where they act in an auto- or paracrine manner [30]. Prostaglandin E_2 (PGE_2) is one of the best known prostaglandins and is generated by the prostaglandin E synthase. These consist of three different forms: two microsomal prostaglandin E synthases and the cytosolic prostaglandin synthase E [31].

The 15-hydroxyprostaglandin dehydrogenase (15-PGDH) belonging to the oxidoreductases family, inactivates all generated prostaglandins by oxidation to 15-keto metabolites which then have greatly reduced biological activity [32].

PG-receptors

The physiological effects of many prostaglandins are mediated by binding to G protein coupled receptors. These effects regulate inflammatory mediations, control hormone regulation, constrict or dilate in vascular smooth muscle cells, and regulate calcium movement and their specific receptors activate signal transduction pathways which could induce chronic processes like angiogenesis. For example, PGE_2 interacts with four cell surface receptors - $EP_{1\text{-}4}$ and the EP_2 receptor subtype is involved in the G_S/cAMP/proteinkinase which is a-signalling pathway leading to an increased vascular endothelial growth factor (VEGF) expression. PGJ_2 and PGA_2 interact with nuclear receptors, belonging to the peroxisome proliferator-activated receptors (PPARs) family. After dimerization with the 9-cis-Retinoid receptor (RXR) and then binding to a sequence specific responsive element located at the promoter of its target gene, they directly induce gene expression [33].

Isoenzymes COX-1 and COX-2

COX-1 is ubiquitary and not a relevant prognostic factor [34]. In contrast, the COX-2 enzyme is not constitutively expressed. The COX-2 gene expression is stimulated by many growth factors, cytokines, and prostaglandins and is associated with inflammation [35]. COX-2 is predominantly a proinflammatory enzyme but late in the inflammatory phase, the enzyme is involved in limiting inflammation.

Studies with COX-1 and COX-2 knockout mice lead to new consolidated findings about the function of these enzymes concerning ovarian functionality and reproduction as well as cardiovascular development [36-39]. The COX enzymes are the main target of non-steroidal anti inflammatory drugs (NSAID) where isoenzymes specifically inhibit the biological activity of COX enzymes. Celecoxib and rofecoxib are selective COX-2 inhibitors whereas acetylsalicylic acid, ibuprofen, and indomethacin are non-specific and target both isoenzymes.

Role of COX-2 in carcinogenesis

The COXs, especially COX-2, play an important role in the development and progression of malignant tumours. The over expression of COX-2 is associated with the differentiation of tumor cells by several mechanisms [40] and can be detected in various epithelial carcinomas such as in colon [41, 42], gastric [43], and esophageal cancers [44], as well as in prostate [45], liver, pancreas, and lung cancers [46]. One of the mechanisms that are modulated during carcinogenesis is neoangiogenesis [47-55].

Epidemiological studies have shown that a continuous intake of NSAIDs protects against the incidence of breast cancer [56-58].

Increased PGE_2 levels can be detected in cultivated human breast cancer cell lines as well as in invasive human breast cancer cells [59-62] and are associated with both a negative hormone receptor status and an escalated metastatic potential [59].

As mentioned previously, PGE_2 is the ligand for at least four cell surface receptors - $EP_{1\text{-}4}$ and several studies have presented the impact of the EP_1-receptor in carcinogenesis of colon and breast cancer [63]. A blockage of the EP_2-receptor leads to a reduction and a diminishment of intestinal polyposis in APC^{D716}-knock-out mice [64]. There was an increased detection of EP_2- and EP_4-receptors in the breast tumors of COX-2-MMTV mice; therefore, it appears that the EP-receptors play an important role in mediating PG functions and in promoting carcinogenesis.

Role of 15-PGDH in carcinogenesis

Increased PGE_2 levels in context to mammary carcinomas are associated with an enhanced cell proliferation, invasiveness, resistance to apoptosis, and angiogenesis [65, 66]. The regulation of plasma PGE_2 level results from its synthesis and its biological inactivation through 15-PGDH, the key enzyme for the biological inactivation of PGs [32]. Recent studies hypothesized 15-PGDH as a tumor suppressor gene in correlation to colon, bladder, and bronchial carcinomas [67-69]. Wolf *et al.* [70] assumed antiproliferative effects of 15-PGDH in breast cancer cells. The estrogen receptor (ER) positive and well differentiated MCF-7 breast cancer cell line had an increased 15-PGDH expression compared to poorly differentiated, ER negative MDA-MB-231 cells, which express COX-2 and lead to primary breast cancer. Different studies reported that MCF-7 cells are the only breast cancer cell line with an enhanced 15-PGDH expression and low levels of 15-PGDH are accompanied by poorly prognostic factors [70]. This data attended by a microarray analysis of van't Veer *et al.* [71] supports the advice that a loss of 15-PGDH expression plays a pivotal role in the development of poorly differentiated mammary carcinomas. Data generated from genetically modified MDA-MB-231 cells that over-express the enzyme and MCF-7

Table 1. — *Immunochemical examinations of COX-2 expression and correlation with selected clinicopathological parameters in breast tissue.*

Reference	N =	COX-2 positive (%)			Correlation of COX-2 expression and clinicopathological parameters						
		Carcinoma	DCIS	Benign tissue	Angio-genesis	HR-status	HER2	Grading	Age	Node +	Big tumor
[77]	44	2/44 (4,5%)	-	-	Not examined						
[78]	27	7/17 (42%)	8/10 (80%)	-	Not examined						
[72]	106	18/42 (43%)	10/16 (63%)	39/48 (81%)	-	No	No	-	-	-	-
[73]	221	80/221 (36%)	-	0%	Yes	Yes	-	Yes	No	Yes	Yes
[79]	46	50%	-	-	Yes	No	No	No	No	Yes	
[74]	1576	589/1576 (37,4%)	-	-	-	Yes	Yes	Yes	-	Yes	Yes
[80]	106	90/106 (85%)	-	-	No	No	No	No	No	No	No
[81]	128	41%	-	-	Yes	-	-	Yes	-	Yes	Yes
[82]	192	40,6%	-	-	-	Yes	Yes	Yes	-	-	Yes
[83]	65	41/65 (63%)	-	-	-	Yes	Yes	-	-	-	-
[76]	43	41/43 (95%)	-	-	-	No	No	Yes	-	-	Yes

Table 2. — *COX-2 mRNA expression and correlation with selected clinicopathological parameters in breast cancer.*

Reference	N =	COX-2-mRNA positive (%)	Clinicopathological correlation of COX-2 with						
			Angio-genesis	HR-status	HER2	Grading	Age	Node +	Big tumor
[86]	40	40/40 (100%)	Not examined						
[72]	9	9/9 (100%)	Not examined						
[87]	7	7/7 (100%)	-	-	-	-	-	-	-
[88]	20	10/20 (50%)	-	-	-	-	-	-	-
[85]	18	18/18 (100%)	Not examined	Yes (PR)	Not examined				
[84]	30	27/30 (90%)	-	Yes (ER+)	-	Not examined			

where 15-PGDH was knockout, corroborates the hypothesis that 15-PGDH acts as a tumor suppressor gene in breast cancer [70]. MDA-MB-231 cells showed a decreased invasiveness similar to studies in colon [67] and bronchial carcinomas [68]. Yan *et al.* [67] reported that 15-PGDH is naturally expressed in colon tissues and was dramatically reduced in colon carcinomas. The reconstitution of 15-PGDH in immunodeficiency mice prevented the colon cancer cells from generating tumors and so the authors concluded, that 15-PGDH acts as tumor suppressor and inhibits the angiogenic and proliferative effects of COX-2 in vivo.

COX-2 expression in breast cancer

Experimental immunochemical studies of COX-2 expression in breast cancer have produced varying and sometimes controversial and inconsistent data. Generally the consensus is that COX-2 is expressed by invasive ductal and lobular carcinoma and that the proportion of COX-2 positive tumors varies between studies (Table 1). In studies where poor prognostic tumor characteristics were examined, a correlation was found between prognostic parameters such as hormone receptor negativity, human epidermal growth factor receptor 2 (HER2) positivity, increased tumor size, high nuclear grade, development of distant metastases, and a reduced survival rate (Table 1) [5]. Moreover COX-2 expression correlates with aromatase expression. An explanation for the variable findings of COX-2 protein expression may be caused by the different scoring systems and cut-offs used for COX-2 immunoreactivity.

Half *et al.* [72] examined immunochemical human breast cell lines of normal and neoplastic breast tissue and detected a COX-2 expression in breast cancer cells in 43%, in DCIS in 62.5% and benign breast cells had a COX-2 expression in 81%. The more elevated COX-2 expression in DCIS in terms of a premalignant lesion might mean that an up-regulation or over-expression of COX-2 occurs relatively early in the carcinogenesis of breast cancer [72]. Contrary to Half *et al.* [72], Denkert *et al.* [73] could not detect a COX-2 expression in benign breast tissue and this may support the partially conflicting data. Denkert *et al.* [73] detected a COX-2 expression in 41% in invasive ductal breast cancer, however detected it in only 14% of invasive lobular tumors and 21% in other breast carcinomas (Table 1). The COX-2 expression was associated with positive axillary lymph nodes (> 50% node positive, just 16% in node negative breast cancer), extensive tumor growth (58% in tumors > 20 mm, in 24% in tumors < 20 mm), poor nuclear grading, vascular invasion, and hormone receptor negativity.

Not all the studies have determined a correlation between COX-2 expression and clinicopathological parameters. Half *et al.* [72] could not demonstrate a significant correlation but Ristimäki *et al.* [74] certainly did show a significant correlation between COX-2 expression and hormone receptor negativity, extensive tumor growth, high nuclear grading, and HER2 positivity. In a recently published paper by Singh-Ranger *et al.* [75], a correlation to distant metastases was described and Nassar *et al.* [76] demonstrated a correlation to nuclear grading and tumor size; however a correlation to important clinical goals such as eradicating the disease and enhancing overall survival rate have not yet been found.

Therefore COX-2 over-expression correlates in a different manner depending upon its aggressiveness the invasive potential of tumor cells, and then consequently exhibiting a higher incidence of distant metastases [40].

Transcriptional studies have also revealed a distinct variation in their results regarding COX-2 expression. The detection rate varies between 50% and 100% in the literature (Table 2).

There is a comparable relationship between COX-2 immunoreactivity and mRNA expression in tumor tissue [77]. Zhao *et al.* [84] demonstrated an increased mRNA expression in hormone receptor positive breast cancer; a result that was confirmed by Singh *et al.* [85] in breast cancer with positive progesterone receptors. However, only a small number of studies have examined the correlation between mRNA expression and clinico-pathological parameters. These results are summarized in Table 2.

These results are contrary to the immunochemically evaluated data, which show an association to hormone receptor negative tumors. This could be explained because before the genetic information of COX-2 is translated into a biologically active protein, COX-2 mRNA is post-transcriptionally modified in the nucleus. Thus, we speculate that the COX-2 mRNA is destabilised by its AU rich sequences and no COX-2 protein is generated. Therefore, the correlation between the hormone receptor status and COX-2 mRNA levels is not obvious in studies where the COX-2 protein expression was investigated [5]. There are some well known factors which affect the COX-2 mRNA levels like interleukin-1 (IL-1) stabilises the highly unstable COX-2 mRNA transcript [89], however steroids may destabilise the COX-2 mRNA [90]. Furthermore, it might be possible that genetically different subtypes of breast cancer express COX-2 and are then associated with both hormone receptor-negative and receptor-positive tumors [91]. Additionally, Ristimäki *et al.* [74] reported that hormone receptor-positive patients who express COX-2 had a poor survival rate.

COX-2 and hormone receptors

There is concurrent evidence regarding the interaction of PGE_2/COX-2 and the ER signalling pathway**.** For example, COX-2 expression is correlated with the expression of the aromatase [92] and in vitro studies support this data. It has been shown that COX-2 promotes the aromatase transcription, whereas COX-2 inhibitors diminish it [93]. Based on the elevated synthesis of prostaglandins in cells that express COX-2, the aromatase expression and activity is increased in breast cells [94, 95]. Expression of aromatase leads to estrogen production and from cell line studies; we know that hormone receptor expression can be induced by sex steroid hormones [96]. All the data supports the close correlation between COX-2 and hormone receptors. Wolf *et al.* [70] reported a link between the estrogen signalling pathway and 15-PGDH by a negative feedback mechanism. High levels of this hormone reduced the 15-PGDH expression but the activity of the estrogen responsive element (ERE) and the activity of the aromatase increased. New studies suggest a synergism between selective COX-2 and aromatase inhibitors.

Results from in vivo studies

The impact of COX-2 in carcinogenesis of breast tumors has been shown in transgenic mice models [97]. It has been reported that the over-expression of COX-2 in breast tissues is associated with decreased BAX and Bcl-xL (pro-apoptotic) and increased Bcl-2 (anti-apoptotic) protein levels. Therefore, the authors suggested that induction of carcinogenesis is COX-2 dependent [97]. In contrast, the resistance to apoptosis is associated with increased COX-2 levels [98]. The importance of COX-2 in correlation to the tumor formation has been investigated in COX-2 knockout mice. The COX-2 knockout mice lead to an 86% reduction of intestinal adenoids [99].

COX-2 and tumorigenesis

The expression of COX-2 is regulated by post-transcriptional and post-translational mechanisms. Different cytokines, growth factors and oncogenes have been shown to induce the COX-2 expression which is associated with carcinogenesis [46, 100].

Influence of COX-2 on angiogenesis and apoptosis

Angiogenesis is the development of new blood vessels and is an important factor in tumor proliferation, invasion, and metastasis. Davies *et al.* [101] showed a significant positive correlation between COX-2 expression and the endothelial surface marker CD31. Other reports confirmed a positive correlation between COX-2 and the VEGF [102, 103]. During carcinogenesis, COX-2 modulates neoangiogenesis and seems to stimulate the production of proangiogenic factors such as VEGF, basic fibroblast growth factor (bFGF), transforming growth factor 1 (TGF1), platelet derived growth factor

(PDGF), and endothelin [104, 105]. The application of selective COX-2 inhibitors decreased the angiogenesis in different vivo models [106]. Recently, an angiogenesis independent mechanism, so called vasculogenic mimicry (VM), was described where poorly differentiated breast cancer cells were nourished without the mechanisms of classic neoangiogenesis [107-109]. VM is a phenomenon of vessel formation of epithelial tumor cells without any participation of endothelial cells and it is a mechanism independent of or simultaneous to neoangiogenesis thus ensuring the tumor perfusion [110]. Hence, VM might be an important factor for new antiangiogenic therapeutical approaches. The existence of VM in breast cancer patients is associated with a poor five-year survival rate compared to patients without. [111]. Basu *et al.* [112] reported that highly invasive MDA-MB-231 breast cancer cells and the less invasive subtype MDA-MB-435 cells that over-express COX-2, formed new micro vessels. In contrast, non-invasive MCF-7- and ZR-75-1-breast cancer cells which had a lower COX-2 expression did not. The application of the COX-2 inhibitor celecoxib (40 und 60 µmol/l, $p > 0.001$), inhibits the formation of new vessels. This effect was restored with PGE_2. This data was confirmed by an in vivo xenograft model. VEGF, growth related protein (GRO), IL-6, IL-8, tissue inhibitor of matrix metalloproteinase (TIMP) 1, and TIMP2 were the main angiogenic proteins which were inhibited by celecoxib [112].

Breast cancer and NSAIDs

The rationale for using NSAIDs is their non-selective (ASS, ibuprofen, etc.) or selective (COX-2 inhibitors such as celecoxib) suppression of the COX-system. In a meta-analysis consisting of 14 epidemiological studies (six cohort studies and eight case control studies), breast cancer risk was reduced by 18% due to constant intake of NSAIDs [113]. An extensive Canadian study including 5,882 patients reported a reduction of breast cancer incidence by 24% due to the NSAIDs intake for two to five years [58]. Another case control study demonstrated a 40% reduction after five years of NSAIDs intake [57]. These results seemingly justify the preventive use of NSAIDs, however, contrary results were delivered by the Nurses Health Study. This trial showed no difference during the intake of ASS (100 mg) in neither women with breast cancer nor in healthy women [114]. On the contrary it was in patients with colon cancer who led the continuous intake of NSAIDs to a reduction of incidence in 40-50% [56, 115, 116].

Data of animal models supports the use of selective COX-2 inhibitors for both therapeutic and preventive uses. For instance, the use of celecoxib in rats led to an averaged downsizing of breast tumor volume by 32%, however, a tumor volume enlargement of 518% was observed in the control group [117]. Harris *et al.* [118] examined the influence of celecoxib in 120 rats. Three groups of rats were formed. In one group the food was enriched with celecoxib. The other two groups obtained either ibuprofen or nothing. After seven days 7,12-Dimethylbenz(a)anthracene (DMBA) was applied intragastrically and the described food was continued for another 105 days. A distinct reduction in tumor incidence, variety, and tumor volume was shown in the celecoxib treated group [118]. In a recently published paper Barnes and co-workers [119] could induce breast tumors in mice by injecting estrogen-positive MCF7/HER2-18- and estrogen-negative MDAMB231 breast cancer cells. The application of celecoxib resulted in a significant growth reduction of the MCF7/HER-18 tumors (58.7%) and the MDAMB231 tumors (46.3%) in comparison to the control group. Therefore, celecoxib dropped the COX-2 expression and enhanced the apoptosis significantly [119]. Yoshinaka *et al.* [120] also showed that the use of celecoxib significantly reduced tumor sizes, increased apoptosis, and that a reduced DNA synthesis in the tumor tissue of mice induced breast carcinomas. Moreover the neoangiogenesis was influenced as VEGF-A-mRNA levels were found to be reduced [120].

COX-2-inhibitors in systemic treatment

Several studies have evaluated the significance of COX-2 inhibitors in combination with systemic treatment. A phase II study observed a clinical benefit of 47.5% for the combination of capecitabine and celecoxib in patients with metastatic breast cancer. The combination was well-tolerated [121].

Recently published data about COX-2 and its significance on the aromatase and influence on the female hormonal balance are of strong interest. Besides finding an increased effect on estrogen synthesis in malignant breast tissue, a strong correlation between COX-2 and aromatase mRNA expression were found. This data supports the assumption that COX-2 is able to regulate aromatase activity in breast tissue [92]. A possible synergism between COX-2 and aromatase-inhibitors is even more interesting and hence a prospective randomised phase III multicenter trial (REACT-trial) was conducted that included primary breast cancer patients in order to evaluate the combination of celecoxib and exemestane, an aromatase inhibitor, in an adjuvant setting. The combination of celecoxib and exemestane was already well-tolerated and had shown a clinical benefit of 74% [122] or had led to a benefit extension (median 96.6 weeks vs. 49.1 weeks) in patients with metastatic breast cancer [123].

Other malignancies were also proven on the benefit of selective and non-selective COX-2 inhibitors in combination with other compounds such as chemotherapy [124, 125], tyrosinekinase inhibitors [126], and other new approaches [127]. Some of them are encouraging, like the results of the ASCENT trial [124] and some are disappointing. Further work is required to establish how NSAIDs can be best applied for therapeutic benefit.

Vitamin D

Vitamin D metabolism

Vitamin D, a secosteroid hormone, is assimilated by food (milk, fish, liver), multi-vitamin preparations, and dietary supplements [128]. Vitamin D is also synthesised from 7-dehydrocholesterol and provitamin D3 after skin exposure with sunlight (ultraviolet spectrum 290-315 nm) [129]. Based on its animal or herbal origin, there are two existing vitamin D metabolites: cholecalciferol (vitamin D_3) and ergocalciferol (vitamin D_2) [17], which is less efficient in increasing the 25-hydroxyvitamin D [$25(OH)_2D_3$] serum levels [130]. Cholecalciferol attains to the liver through the bloodstream and is transformed to $25(OH)_2D_3$ (25-hydroxyvitamin D_3, calcediol, 25-hydroxycholecalciferol) by a hydroxylation on the C25 position [131, 132]. $25(OH)_2D_3$, a circulating metabolite, correlates with the vitamin D balance. The hydroxylation of cholecalciferol on the C25 position is inadequately regulated. $25(OH)_2D_3$ level increased with the vitamin D intake, therefore, the $25(OH)_2D_3$ serum level is normally used as an indicator of the vitamin D balance [133]. The serum level range of $25(OH)_2D_3$ is between 10 and 50 ng/ml and round about 30 pg/ml for $1,25(OH)_2D_3$ [134]. $25(OH)_2D_3$ is renally converted to the biologically active metabolite 1,25-dihydroxycholecalciferol [$1,25(OH)_2D_3$] by the 1-a-hydroxylase (CYP27B1). $1,25(OH)_2D_3$ is 100-1,000 fold more active than the other natural metabolites [135]. The 1-a-hydroxylase, a mitochondrial enzyme, which belongs to the P450 enzyme family, is located in the renal proximal tubule. Besides the renal expression of the enzyme, many studies reported an extra-renal expression of 1-a-hydroxylase and thus an extra- renal synthesis of $1,25(OH)_2D_3$. This enzyme has been detected in many cell types and tissues, e.g. prostate, breast, lung, pancreas, parathyroid, and monocytes [136]. The extra-renal synthesised $1,25(OH)_2D_3$ has cell specific functions and as a result acts as local auto- and paracrine factors. In this context, many extra-renal effects of $1,25(OH)_2D_3$, e.g. cell cycle arrest, induction of apoptosis, and cell differentiation, have been reported [136]. The fine tuned activity of 1-a-hydroxylase correlates inversely with the calcium metabolism and thus the circulating levels of $1,25(OH)_2D_3$ correlates inversely with the ingested amount of calcium [137]. $1,25(OH)_2D_3$ serum levels are maintained in pmol/l range by a classic negative feedback mechanism. The decrease of calcium or phosphate levels leads to an increase of the 1-a-hydroxylase activity and an enhanced synthesis of $1,25(OH)_2D_3$ which in turn promotes the intestinal resorption of calcium and phosphate and the calcium mobilisation from the bones. The activity of 1-a-hydroxylase decreased with increasing $1,25(OH)_2D_3$ levels, which leads to 24 hydroxylase activation. This enzyme degrades $1,25(OH)_2D_3$ to its inactive metabolite $24,25(OH)_2D_3$ [138, 139], which is subsequently converted to calcitroic acid and excreted. Hence, the nutritive intake of calcium directly regulates 1-a-hydroxylase activity and indirectly modifies parathormone levels. This hormone produced in the parathyroids increases the phosphate excretion in the proximal tubule but promotes sodium, potassium, and calcium resorption in the distal tubule. Under normocalcaemic conditions, the activity of 1-a-hydroxylase is inhibited. These regulations are necessary to synthesize $1,25(OH)_2D_3$ although much is needed to cover the calcium and phosphate demand and to avoid a $1,25(OH)_2D_3$ intoxication [139]. The circulating vitamin D level depends on many different factors such as: the vitamin D content in either the ingested nutrition or the dietary supplements, and the endogenous production and degeneration via vitamin D metabolising enzymes. A simplified scheme of vitamin D metabolism is presented in Figure 2.

Extra-renal vitamin D metabolizing enzymes

The biologically active metabolite is produced after a series of hydroxylations through cytochrome P450 enzymes which belong to the cytochrome p450 super family. The different enzymes are handled as follows:

1-a-hydroxylase (CYP27B1)

The 25-hydroxyvitamin-D_3-[$25(OH)_2D_3$]1-a-hydroxylase (1-a-hydroxylase) is encoded by the CYP27b1 gene and catalyzes the synthesis of $1,25(OH)_2D_3$ from $25(OH)_2D_3$. $1,25(OH)_2D_3$ is the most important regulator of the enzyme that leads to a decreased enzyme expression. The regulation of the extra renal 1-a-hydroxylase depends on local factors like cytokines (ILs, interferones, and tumor necrosis) and growth. The optimal $1,25(OH)_2D_3$-level tuning mechanism is not yet completely understood [139]. The reduced expression of the enzyme suggests the involvement of a negative vitamin D responsive element (VDRE) and Turunen *et al.* [140] showed that the enzyme's response to $1,25(OH)_2D_3$ is a cell specific event with participation of many VDREs. The suppression of cell proliferation, the induction of apoptotic events, and the modulation of immune responses are counted among the classical features of $1,25(OH)_2D_3$. After binding to the vitamin D receptor, $1,25(OH)_2D_3$ is able to arrest the cell cycle of a tumor cell in the G1-G0 phase via specific mechanisms [139]. In prostate and colon cancer the tumor protective effects of vitamin D is correlated to vitamin D deficiency [141]. Much data reports that both the renal and extra renal 1-a-hydroxylase are based on the expression of the same gene product. In contrast to the renal 1-a-hydroxylase, the extra-renal enzyme is not subjected to the autoregulation as mentioned above [136, 142]. Therefore the enzyme's tissue specific expression might be a key mechanism in connecting the vitamin D metabolism to the anticarcinogenic effects of $1,25(OH)_2D_3$.

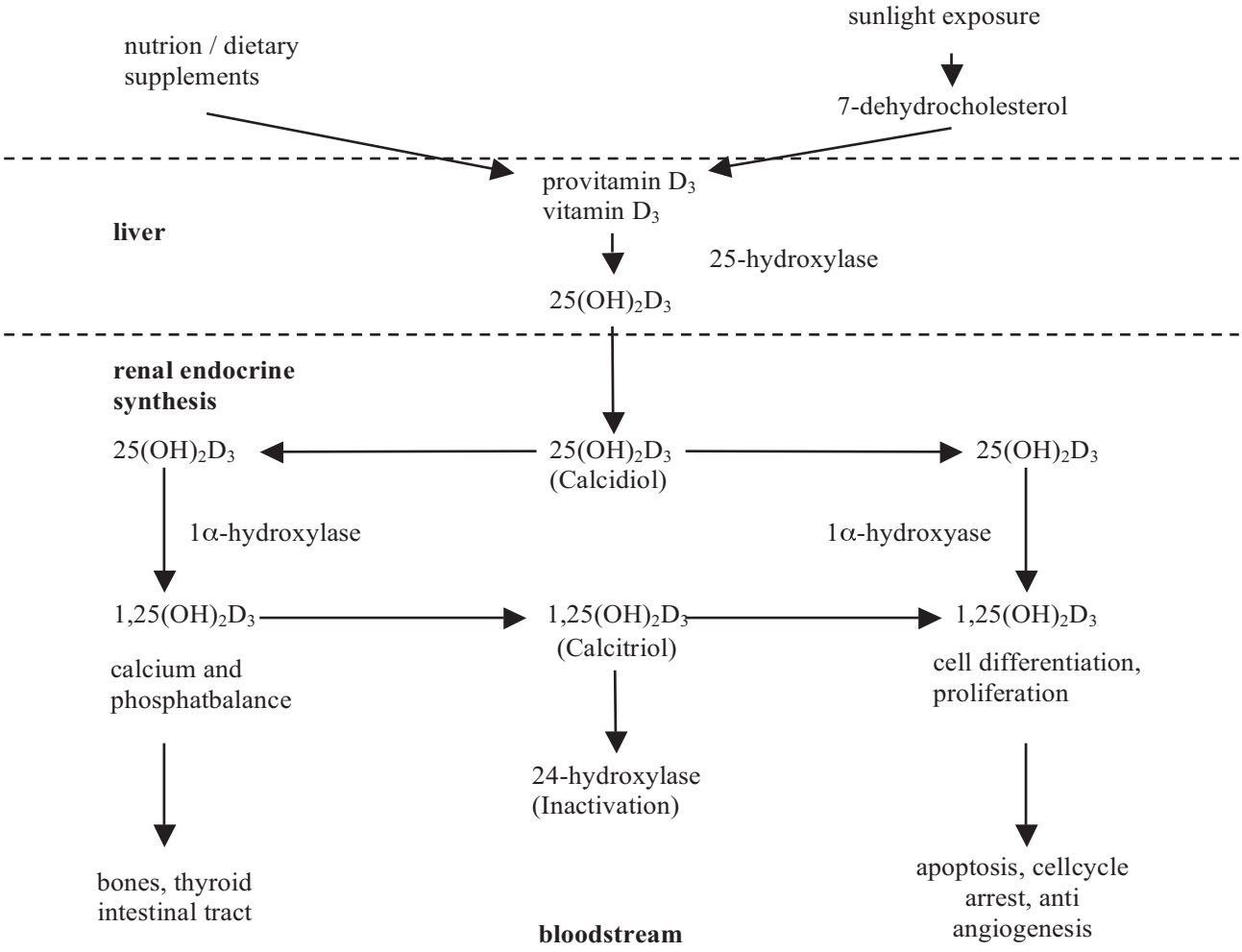

Figure 2. — Simplified scheme of vitamin D metabolism. Vitamin D (food intake, synthesis in skin) is metabolised in liver to $25(OH)_2D_3$, then via the renal 1-a-hydroxylase (endocrine signalling pathway or extra-renal in tissues (autocrine/paracrine signalling pathway) to $1,25(OH)_2D$.

Although the enzyme's cytokine and growth factor related regulation is not completely understood, it has been shown that different cytokines stimulate the 1-α-hydroxylase in different cell types [139, 143-146]. Another potential mechanism of gene regulation is the incidence of different gene polymorphisms [147] and inactive variants due to alternative splicing of the 1-a-hydroxylase mRNA, but this mechanism's function is not completely clarified. Alternative splicing within the post-transcriptional modification is a normal process of gene expression in breast cancer cells and based on the pre mRNA, different mature mRNAs are generated when introns or exons are deleted or added. Thus, the translation of these mRNAs leads to different enzyme proteins, however, mis-spliced mRNAs are usually quickly degraded although it appears that this mechanism has failed in various cells. It has been reported that different protein variants of 1-a-hydroxylase might have diverse biological functions. Fischer *et al.* [148] showed six different variants of the enzyme in MCF10F via nested touchdown polymerase chain reaction (PCR), but in MCF-7, these variants appeared weakly expressed. Based on this data, the authors concluded that because alternative splicing regulates the level of the active enzyme extra-renally, it therefore regulates the local production of $1,25(OH)_2D_3$ [149]. The activity of the extra-renally expressed 1-a-hydroxylase is an important factor of the tumor pathophysiology because of an accumulation of $1,25(OH)_2D_3$ in many tissues. Studies of prostate [150, 151], colon [152-154], and breast cancer [148, 155, 156] have shown the expression of 1-a-hydroxylase in healthy as well as in malignant tissues. Thus, $1,25(OH)_2D_3$, which is produced extra-renally might have autocrine behaviour to protect cells against transformation and supports the suggestion of its carcinoprotective effects. Accordingly, low 1-a-hydroxylase levels correlate with the risk of prostate-, colon- [157] or breast cancer [158, 159]. Moreover, the extra-renal production of $1,25(OH)_2D_3$ inhibits cell proliferation and

promotes cell differentiation in xenograft models [160]. Besides the expression of the 1-a-hydroxylase in breast [155, 161], endometrial [162], cervical, and ovarian carcinomas [163], the induction of the enzyme has also been shown in lymphomas [164] and dysgerminomas [165]. In these reports, the local synthesis of $1,25(OH)_2D_3$ was mediated by the 1-a-hydroxylase expression of tumor associated macrophages. The expression of the enzyme mammary gland tissue occurs in lobules and ductus, primarily in the cancer tissue and invasive tumor cells and inflammatory infiltrate. Thus, it might be possible that the enzyme activity and the vitamin D receptor (VDR) expression are considerably higher than in the benign tissue compared to aggressive tumor cell lines (MCF-7res, MDA-MB231) [166]. Townsend *et al.* [166] compared breast cancer and benign tissue samples via reverse transcription PCR. They reported a 27-fold induction of the 1-a-hydroxylase expression and sevenfold induction of the VDR expression in tumor samples. Because 80% of the tumor tissues had an increased 1-a-hydroxylase and VDR, they concluded that there was a closed coupling of both gene products. These results are in compliance with Segersten *et al.* [161]. The capacity of 1-a-hydroxylase to synthesize $1,25(OH)_2D_3$ within the mammary gland parenchyma results in, on the one hand, the available amount of $25(OH)_2D_3$, and is dependent on sunlight exposure and the season [167-170] – normally there is no definite correlation between $25(OH)_2D_3$ and $1,25(OH)_2D_3$ – yet on the other hand, the level of the extra-renal production of $1,25(OH)_2D_3$ is limited by the expression of the $1,25(OH)_2D_3$ – decomposing enzyme 24-hydroxylase, which is stimulated by $1,25(OH)_2D_3$ in VDR expressing tissues. Based on the missing correlation of 24-hydroxylase and VDR or the 1-a-hydroxylase in breast cancer tissues, it seems that 24-hydroxylase is independently regulated. Kemmis *et al.* [171] demonstrated the expression of a functioning VDR and an inhibition of proliferation via $1,25(OH)_2D_3$ in benign breast cells and MCF-7. The VDR expression in human mammary epithelial cells (HMEC) breast cells was higher than in MCF7 cells. Furthermore, the authors showed an expression of $25(OH)_2D_3$ metabolizing 1-a-hydroxylase and 24-hydroxylase in these cell types, whereas the 1-a-hydroxylase expression was higher in MCF-7. In contrast to renal HKC8 cells, the expression of 1-a-hydroxylase was not inhibited by $1,25(OH)_2D_3$. Based on the strong induction of the 24-hydroxylase through the $1,25(OH)_2D_3$ application, the authors showed that MCF7 cells were more sensitive in response to $1,25(OH)_2D_3$ compared to HKC-8 and HMEC cells. From this data, they concluded that there is a functional vitamin D receptor as well as intact signalling transduction pathways in MCF-7 cells. The data suggests that the synthesis of $1,25(OH)_2D_3$ and the activation of the VDR inhibits the cell proliferation in breast cells. Thus, the treatment of benign breast cells with $25(OH)_2D_3$ leads to an activation of the VDR transcription and the regulation of its target genes (CYP27B1, CYP24), and finally to an inhibition of cell proliferation. According to that, CYP27B1 lords it over CYP24 which means a transformation of $25(OH)_2D_3$ to $1,25(OH)_2D_3$. Kemmis *et al.* [171] have shown for the first time that physiological $1,25(OH)_2D_3$ levels (30-100 nmol/L) are able to inhibit cell proliferation in benign HMEC cells and in MCF7 breast cancer cells. Interestingly, aging process and the associated lack of estrogens correlate with decreased $25(OH)_2D_3$ levels. The reason is that the ability of estrogen to stimulate the renal CYP27B1 activity [172]. Accordingly, the lack of estrogens leads to decreased $1,25(OH)_2D_3$ levels and presents the highest risk for breast cancer in postmenopausal women [273].

24-hydroxylase (CYP24)

The 25-hydroxyvitamine D_3-24 hydroxylase (24-OHase, 24-hydroxylase) encoded by the CYP224 gene is induced by $1,25(OH)_2D_3$ in breast cell lines where the enzyme is time and dose dependently stimulated by $1,25(OH)_2D_3$ [174]. An increased enzyme expression in ovarian, cervical, and breast cancer compared to healthy tissue samples has been shown by immunochemistry and real time PCR [163]. In contrast, Townsend *et al.* [166] showed a four-fold increase of the enzyme expression in malignant breast tissues compared to healthy tissue samples using the same technique. Additionally, the expression of 24 hydroxylase increased in breast cancer cells foremost in hormone resistant MCF-7 Res and the aggressive MDA-MB231 cells compared to benign MCF-12A cells [166]. Kemmis *et al.* [171] reported the highest 24 hydroxylase expression in MCF-7 cells and Segersten *et al.* [161] showed a two-fold enzyme expression in tumor tissues compared to benign tissue samples. The authors concluded that the conversion of $1,25(OH)_2D_3$ into the inactive metabolite $1,24,25(OH)_2D_3$ is significantly higher in malignant tissues. Furthermore Townsend *et al.* [166] detected the enzyme only in breast cancers with an increased 1-a-hydroxylase and VDR expression. Further analysis showed that in a healthy tissue sample expression of 24-hydroxylase correlated with both 1a-hydroxylase and VDR. There was no such correlation in breast tumors. Hypothetically, the 24 hydroxylase acts as a part of a well-organized feedback mechanism and is transcriptionally modulated to increase the local $1,25(OH)_2D_3$ and VDR level [166]. The synthesis of $1,25(OH)_2D_3$ via the 1-a-hydroxylase has been shown in benign and malignant mammary gland tissues but this mechanism's efficiency in tumor tissues might be affected by a dysregulated 24 hydroxylase expression.

Vitamin D-receptor (VDR / mVDR)

The vitamin D receptor (VDR) is an ubiquitary expressed steroid hormone receptor. Like other steroid, thyroid, and retinoid receptors, the VDR is a member of the nuclear hormone receptor family. The receptor binds to its ligand $1,25(OH)_2D_3$, interacts with other receptors by dimerization, and binds as homodimers or heterodimers to specific DNA

sequences. So called VDRE recruit additional co-activators (such as SRC-1, GRIP-1/TIF2, ACTR) and interact with the transcriptional processing order to initiate or inhibit the transcription of its target genes [25]. It is well known, that steroid receptors consist of different variants with distinct specificities. Sunn *et al.* [175] described an N-terminal variant of the VDR. $1,25(OH)_2D_3$ mediates its genomic effects as a VDR ligand and via the directed binding to the VDRE [176]. Besides its function in bone metabolism and in the calcium/phosphate balance, the VDR interacts with different signalling pathways, e.g. with p21, a cyclin dependent kinase inhibitor which is involved in cell cycle regulation and inhibition of the cancer cell proliferation [26]. There are some suggestions about the existence of a membrane VDR (mVDR) [177]. The mVDR mediates its signals through the change of the intracellular calcium concentrations and through interactions with the protein kinase C and enzymes of the mitogen-activated protein kinases (MAPK) family [178-183]. Although the mVDR seems unrelated to the nuclear VDR, Marcinkowska *et al.* [184] reported an interaction of both receptors. The function of this mechanism is not clearly defined and the cloning of the mVDR has failed until today.

Many studies reported that extra-renal VDR expression is associated with the non-classical effects of $1,25(OH)_2D_3$. The VDR expression has been shown in healthy breast tissues and in more than 80% of the breast cancer tissues [185]. The natural ligand of the VDR, $1,25(OH)_2D_3$ and many new developed synthetic vitamin D analogues inhibit cell proliferation and induce apoptosis in breast cancer cell lines [186, 187]. Furthermore, in animal models, vitamin D analogues retard the tumor growth and lead to a regression of breast tumors [12].

Vitamin D-receptor gene polymorphism

The gene that encodes the VDR has various polymorphisms. It has been hypothesised that the genetic VDR polymorphism influences the breast cancer risk due to its potential effects on VDR gene expression and protein function [188, 189]. Many polymorphisms of the VDR gene have been identified and several, such as FOK1, Bsm1, APA1, TAQ1, and Poly(A) are well analysed [190, 191]. The studies that were conducted had conflicting results [191]. Curran *et al.* [192] showed a significant association of the VDR polymorphism APA1 and TAQ with the breast cancer risk. A significant increased breast cancer risk in women with the ff genotype FOK1 was observed by Chen *et al.* [193]. Sinotte *et al.* [194] detected a significant link between familial breast cancer disposition and FOK1. Other data came from Trabert *et al.* [195] who found a correlation between a higher breast cancer risk and the genotype Bsm1 bb in postmenopausal women, although there is also published data without any evidence for a link between VDR polymorphisms and breast cancer risk [196-199]. An analysis of the last 13 published studies in which different VDR polymorphisms and its relation to breast cancer were examined, leads to the suggestion that the modification of breast cancer risk is associated with certain VDR polymorphisms and therefore $1,25(OH)_2D_3$ might modify the risk of breast cancer [200]. A recently published paper by McCullough *et al.*[201] presented certain VDR gene polymorphisms associated with a decreased breast cancer risk in women who ingested high doses of calcium (no calcitriol), concluding that nutritive influences might modify the link between gene polymorphisms and breast cancer. This data could shed light on breast cancer risk evaluation or could even be used in a predictive manner to answer the question about which women are strongly endangered to develop distant metastases.

Calcium

Like vitamin D, humans ingest calcium through food or dietary supplements; 99% of calcium is bound as hydroxyl phosphatide in bones and teeth [202]. Only 1% calcium is extracellularly located. Plasma levels of calcium (Ca^{2+}) are limited by intestinal absorption, renal secretion, and reabsorption. Additionally, the skeletal calcium storage and resorption keep the plasma levels of calcium in a closed range (3.5–5 mmol/L) [202].

Vitamin D, calcium, and breast cancer risk

Dietary and supplemental vitamin D intake

For $1,25(OH)_2D_3$ several studies have shown both an antiproliferative effect and an inhibition of angiogenesis in malignant and healthy breast cancer cells [17, 185, 203-206]. In mouse models, an increased intake of vitamin D led to the suppression of epithelial hyperproliferation and tumorigenesis of the mammary gland that was caused by rich nutrition [207, 208].

Last but not least, it has been proven by the First National Health and Nutrition Examination Survey Epidemiologic Follow-Up Study that sunlight exposure is inversely correlated with breast cancer risk [209, 210]. In this study, the female population in the north-eastern parts of the U.S. have a higher risk of contracting breast cancer compared to the other states of the U.S. This leads to the suggestion that sunlight induced vitamin D production has a positive influence in avoiding breast cancer [20].

In contrast, in the Nurses' Health Study, there was an inverse association between vitamin D intake and breast cancer risk among premenopausal women, but no association among postmenopausal women [20]. Consistent with this observation, a study published a few years ago was based on the Cancer Prevention Study II Nutrition Cohort and observed no

associations between breast cancer and total and dietary vitamin D intakes among postmenopausal women [211]. Another Italian study recently showed an inverse association between vitamin D intake (in the study > 143 IU) and breast cancer in 2,569 breast cancer patients [212]. Two other studies that concentrated on vitamin D deficiency and its susceptibility for breast cancer incidence approved that a deficiency conditional on nutrition in adolescence does not lead to an increased breast cancer risk [213, 214].

The proper dose of vitamin D remains unclear and a recommendation does not exist, however a meta-analysis gives evidence towards a dose of > 400 IU per day to reduce breast cancer risk [213].

Role of vitamin D in breast cancer

To date, there have been several epidemiologic studies of the association between vitamin D and breast cancer risk, however, their results have not been consistent. Several studies observed an association between $25(OH)_2D_3$ plasma levels and breast cancer incidence [19, 215-217]. The predictive value of $25(OH)_2D_3$ plasma levels depends upon the time they have been measured. Plasma levels that have been measured within a few years before breast cancer diagnosis are less predictive than plasma level measured many years before [217]. Furthermore, plasma levels that have been measured around 15 years before diagnosis do not have any aetiological value for the genesis of breast cancer [216].

Bertone-Johnson *et al.* [217] found a marginally significant reduction of breast cancer risk in women > 60 years who had elevated $25(OH)_2D_3$ and $1,25(OH)_2D_3$ plasma levels. In contrast, published data by Shin *et al.* [20] demonstrated a significantly decreased breast cancer incidence in premenopausal, but not in postmenopausal women, who had continuous vitamin D intake.

Furthermore, a case control study observed that women with plasma $25(OH)_2D_3$ concentration <5 0 nmol/L had > five times higher risk of breast cancer than those with plasma concentrations exceeding > 150 nmol/l [158]. Janowsky *et al.* [19] also showed an inverse association between $1,25(OH)_2D_3$ plasma levels to the point of diagnosis and breast cancer risk in patients with breast cancer. However, there was no difference in $1,25(OH)_2D_3$ plasma levels between patients with breast cancer and those with DCIS. The authors suggested that the grade of invasion was not correlated with the extent of $1,25(OH)_2D_3$ level. Another nested case-control study with 96 breast cancer cases and 96 controls found no association between prediagnostic $1,25(OH)_2D_3$ levels and levels at the time of diagnosis and breast cancer risk among postmenopausal women [216].

The circulating concentration of $25(OH)_2D_3$ is considered to be an excellent measure of the availability of vitamin D from the diet, supplements, and from synthesis in the skin [218]. Its potential importance in breast carcinogenesis is due to the fact that $25(OH)_2D_3$ can be metabolised to $1,25(OH)_2D_3$ by 1-a-hydroxylase in breast tissue [155]. Thus, $25(OH)_2D_3$ levels may be more representative of intracellular levels of $1,25(OH)_2D_3$ than circulating levels of $1,25(OH)_2D_3$ [217]. To date, no studies have been published investigating intracellular or tissue levels of $1,25(OH)_2D_3$ and $25(OH)_2D_3$ in association with breast cancer risk.

Dietary and supplemental calcium intake

Many studies about the importance of calcium and its association to breast cancer have already been published. Most of them are case-control studies and nearly all of them are relatively small, and there is insufficient documentation regarding risk factors for breast cancer in multivariate analyses.

Calcium is participating on carcinogenesis via regulation of cell proliferation, differentiation, and apoptosis [219-221]. Cell proliferation and differentiation of breast cells can be increased by elevated calcium levels [208, 222, 223]. Boyapati *et al.* [224] observed a non-significant inverse association between calcium intake and breast cancer risk among pre- and postmenopausal women but the Nurses' Health Study has shown this association only for premenopausal women [20].

The anti-carcinogenic effects of calcium are last but not least, mediated by vitamin D, therefore calcium is one of the key mediators of the vitamin D induced apoptosis in breast cancer cells [208].

Calcitriol and prostaglandins in cancer

The stimulation of the renal calcitriol $[1,25(OH)_2D_3]$ synthesis in vitro is well known as well as the inhibition of acetysalicylic acid as a non-selective NSAID [225]. This justifies the clinical use of NSAIDs in treating arthritis for example. Hayes *et al.* [226] observed an inhibition of calcitriol synthesis caused by PGE_1 and PGE_2 in synovial fluid macrophages from arthritic joints and with that they proved the link between vitamin D and prostaglandin metabolism. Several published studies have proven the anti-carcinogenic effects shown in different signalling pathways on prostate cancer cells [227-229]. The team around David Feldman examined the influence of calcitriol in established human prostate cancer cell lines (androgen dependent LNCaP cells and androgen independent PC-3 cells) and in primary normal prostatic epithelial cells derived from normal and cancerous human prostate tissue. They showed that calcitriol

regulates biologically active prostaglandin levels and prostaglandin actions by three mechanisms: calcitriol suppresses the COX-2 expression and moreover it up-regulates the expression of 15-PGDH. This dual influence of calcitriol was associated with a decrease of PGE_2 secretion in prostate cancer cells. Calcitriol reduces the mRNA expression of prostaglandin receptors EP_2 and FP, additionally a mechanism to inhibit the biological activity of prostaglandins.

The combination of calcitriol and NSAIDs led to a significant growth inhibition in prostate cancer cells via its synergistic effects. These findings might postulate that calcitriol and NSAIDs are definitely a useful combination in chemo preventive and/or therapeutic strategies in prostate cancer [230]. Unpublished own data support these results as we showed an inverse correlation between VDR- and COX-2 expression in breast cancer cells and a downregulation of COX-2 and an upregulation of 15 PGDH by calcitriol. Therefore we propose that these findings and suggest a possible link between VDR, associated target genes and the prostaglandin metabolism.

Concluding remarks

In conclusion, there is promising preclinical data inhibiting COX-2 in breast cancers, therefore the chance exists to innovatively disturb carcinogenesis of those gynecological oncological neoplasms Phase II trials have already been conducted to clear the safety of a celecoxib treatment in metastatic breast cancer. Furthermore, calcitriol and calcium have shown anticarcinogenic effects in experimental studies and several epidemiological studies have demonstrated an inverse association between vitamin D and calcium intake and breast cancer. Other studies have detected an inverse association between plasma and serum levels and breast cancer risk. Experimental studies support the hypothesis that the reduction of breast cancer risk is more significant among premenopausal women than among postmenopausal women and microsomal prostaglandin E synthase-1 (mPGES-1) and EP receptors might be important targets for the development of new anti-inflammatory and anti-proliferative tumor therapies.

Questions that remain unanswered are: has calcitriol as antiproliferative effects in breast cancer as was proven in prostate cancer? Does a link exist between vitamin D and prostaglandin metabolism in breast cancers? These questions have to be answered as the increasing incidence of breast cancer have yet to be solved. Innovative treatment strategies fall on fruitful ground. Thus we need further studies that elucidate the importance of COX-2 inhibitors in the preventive as well as in the adjuvant settings in breast cancers and finally that will evaluate the promising importance in the neoangiogenesis in detail.

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